

## **Performance Monitoring Protocol (QA/QC) for the Thermo LTQ OrbiTrap XL LC/MS (ESI)**

### **1 Scope**

This document addresses the performance monitoring (QA/QC) of the Thermo LTQ OrbiTrap XL LC/MS system consisting of a Thermo Electron LTQ OrbiTrap XL Mass Spectrometer (MS), and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, and Chemistry Unit general physical and chemical analysis.

### **2 Principle**

The LTQ OrbiTrap XL system is comprised of a Shimadzu Liquid Chromatograph (LC) and a Thermo Electron Linear Ion Trap LTQ MS coupled to an OrbiTrap XL High Resolution MS. This system can be used for both unit mass and high resolution accurate mass chemical analyses. The instrument is configured with an API source that is capable of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). The instrument is primarily used in ESI mode. However, this protocol can also be used for APCI and APPI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

### **3 Equipment/Materials/Reagents**

- a. Instrumentation - Thermo Electron LTQ MS, OrbiTrap XL, API Source, Shimadzu Prominence LC, and Data System with XCalibur software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Ion Trap Gas - Helium, 99.99% (high purity or equivalent)
- d. Methanol, Optima grade or equivalent
- e. Deionized Water, 18 M $\Omega$ ·cm Milli-Q or equivalent
- f. Acetonitrile, HPLC grade or equivalent
- g. Ammonium Formate, reagent grade
- h. Formic Acid, reagent grade
- i. LTQ ESI Positive Ion Calibration Solution (Thermo or equivalent)

- j. LTQ ESI Negative Ion Calibration Solution (Thermo or equivalent)
- k. Ammonium Hydroxide (NH<sub>4</sub>OH), reagent grade
- l. Codeine (Sigma or equivalent)
- m. Brucine (Sigma or equivalent)
- n. Reserpine (Sigma or equivalent)
- o.  $\gamma$ -Aminobutyric Acid (GABA), (Sigma or equivalent)
- p. Volumetric glassware
- q. Infusion Syringe - 10 to 500  $\mu$ L LC syringe (Hamilton or equivalent)

## 4 Standards and Controls

### 4.1 Testmix

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record all preparations in the Reagent Log. To prepare:

- a. Stock Solution - Weigh 1.5 mg GABA, 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled up.
- b. Testmix Solution - Pipet 4.0 mL of the Stock Solution into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled up.

### 4.2 Calibration Solution

The calibration solution is used for coarse tuning and calibrating both the LTQ and the Orbitrap XL over the entire mass range. This procedure only needs to be performed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

The calibration solution is purchased from Thermo Fisher Scientific or equivalent.

## 5 Calibration

The calibration procedure should be performed as needed, when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

- a. Load a 250  $\mu$ L syringe with the appropriate calibration solution.
- b. Connect the infusion syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10  $\mu$ L/minute.
- d. Load the tune file "calibration\_solution\_positive" (or equivalent).
- e. Check that instrument is in POSITIVE ION mode and collecting CENTROID data.
- f. Set the detector using the parameters listed in the 'Instrumental Conditions' section of this protocol.
- g. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- h. Engage the ESI probe and turn on the MS.
- i. To perform an accurate mass calibration for the OrbiTrap only, open the 'Calibrate' dialog box in Tune Plus, choose the 'Semi-Automatic' tab and check **ONLY** 'Mass Calibration' for FT and then 'Start.'
- j. To perform a unit mass calibration for the LTQ only, open the 'Calibrate' dialog box in Tune Plus, choose the 'Semi-Automatic' tab and check **ONLY** 'Select All - Ion Trap' and then 'Start.'
- k. When the calibration is complete, it will display whether or not the calibration was successful.
  - If the procedure fails, repeat the calibration.
  - When the procedure passes, print the report and evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the spectrum of the calibration solution.
- l. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

## 6 Sampling or Sample Selection

Not applicable.

## 7 Procedures

### 7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use the Xcalibur program (menu: actions > check disk space) to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain. If analysis consists of multiple samples in a sequence, ensure that there is additional sufficient storage space.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 250 p.s.i. remaining.
- c. Record the line pressure of the building helium supply (ion trap gas). The regulator should read between 20 and 40 p.s.i. (30 – 60 p.s.i. if two instruments will be run off the same regulator). If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- d. Check the oil level in the vacuum pumps housed in the compartment directly below the LTQ. If a significant amount of oil is present in the mist filter, then ballast pumps individually by temporarily rotating the ballast switch until the oil has been evacuated.
- e. Check the vacuum pressure to ensure that no significant leaks are present in the system. Do not use if the convectron gauge reads above 2 torr, or the ion gauge (if present) reads above 20 microtorr.
- f. Prime each LC solvent line to be used that day. Open the prime valve on the front of each pump module to be used by turning the valve handle ninety degrees and press the PURGE button on the module. If the pump does not start priming, disengage remote control by pressing the PUMP button and then pressing the PURGE button again. After the prime cycle finishes (about three minutes), close the prime valves.
- g. Prime the autosampler solvent wash by pressing the PURGE button on the front of the autosampler module, then rinse the autosampler needle by pressing the RINSE button.

- h. Verify that the instrument has the correct source probe installed (ESI), the correct tune file loaded (testmix\_pos or equivalent), positive ion mode selected, and centroid data being collected. If a column is installed, remove it from that system and replace it with a zero-dead-volume union.
- i. Select the proper analyzer. In Tune Plus, select 'Ion Trap' analyzer for unit mass resolution using the LTQ. For accurate mass analysis using the Orbitrap XL, select 'FTMS' analyzer and a resolution of 60,000.
- j. Perform an analysis of the testmix prior to the analysis of case samples. For targeted analytes, a positive control can be substituted for the testmix. For testmix analysis, use parameters listed in the 'Instrumental Conditions' section of this protocol. Start the HPLC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'TMymmdd' (or equivalent). Make three 5  $\mu$ L injections of the testmix solution at least 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and spectra for components in the testmix.
- k. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

## 7.2 As Needed Checks

- a. Re-cut or replace the sample capillary as needed.
- b. Clean or replace the heated capillary as needed.
- c. Clean the ion sweep cone (the heated interface front plate) as needed.

## 8 Instrumental Conditions

### 8.1 Testmix

#### Liquid Chromatograph

Mobile Phase:	From discipline specific SOP
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	5 $\mu$ L

#### Mass Spectrometer

Ionization:	ESI
Tune File:	testmix_pos
Sheath Gas Flow:	14 (arb)
Aux Gas Flow:	3 (arb)

Sweep Gas Flow: 3 (arb)  
 Scan Mode: Full Scan  
 Scan Range: 100-650 m/z

## 8.2 Calibration

### Mass Spectrometer

Ionization: ESI  
 Tune File: calibration\_solution\_positive  
 Scan Mode: Full Scan  
 Scan Range: 100-2000 m/z

## 9 Decision Criteria

### 9.1 Testmix: Unit Mass

Verify the results of the testmix. The following ions should be observed in the three testmix injections when using the system for unit mass resolution. RICs should show contemporaneous signals for components at the following masses:

Caffeine	195 m/z
Codeine	300 m/z
Brucine	395 m/z
Reserpine	609 m/z

### 9.2 Testmix: Accurate Mass

When using the OrbiTrap analyzer for accurate mass analysis, the testmix components should be observed within  $\pm 3$  mmu of their expected monoisotopic masses:

	<u>Formula</u>	<u>Expected Mass</u>	<u>Acceptable Mass Range</u>
Caffeine	C <sub>8</sub> H <sub>11</sub> O <sub>2</sub> N <sub>4</sub>	195.0877	195.0847 - 195.0907
Codeine	C <sub>18</sub> H <sub>22</sub> O <sub>3</sub> N	300.1594	300.1564 - 300.1624
Brucine	C <sub>23</sub> H <sub>27</sub> O <sub>4</sub> N <sub>2</sub>	395.1965	395.1935 - 395.1995
Reserpine	C <sub>33</sub> H <sub>41</sub> O <sub>9</sub> N <sub>2</sub>	609.2807	609.2777 - 609.2837

### 9.3 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

Caffeine	195 m/z
MRFA	524 m/z
Ultramark	1022 m/z
	1122 m/z
	1222 m/z

1322 m/z  
1422 m/z  
1522 m/z  
1622 m/z  
1722 m/z  
1822 m/z  
1922 m/z

## 10 Calculations

Not applicable.

## 11 Measurement Uncertainty

Not applicable.

## 12 Limitations

This procedure is specific to positive-ion analyses. The LTQ OrbiTrap XL system should not be switched to negative ion mode without the assistance of appropriate instrument support personnel.

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

## 13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

## 14 References

Manufacturer's Instrument Manuals for the specific models and accessories used (electronic or hardcopy).

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and*

*Systems Support SOP Manual.*

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

"Preparation of Chemical Reagents" (Tox 103) *Toxicology SOP Manual.*

"Solid Phase Extraction of Opioids from Biologicals with Analysis by LC-Tandem MS" (Tox 418) *Toxicology SOP Manual.*

*FBI Laboratory Safety Manual.*

Rev. #	Issue Date	History
0	07/06/09	New document
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Removed Waters Alliance LC from Sections 2 and 3 a. Added 'appropriate instrument support personnel' to Sections 5 l, 7.1 b, c & k, and 12. Updated heading in Section 6. Added targeted analytes to Section 7.1 j. Changed to discipline specific SOP in Section 8.1. Reduced decimal places from five to four in Section 9.2. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

### **Approval**

Redacted - Signatures on File

Drug Chemistry/  
General Chemistry  
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Date: 09/28/2018

Toxicology  
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### **QA Approval**

Quality Manager:

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